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Protective effect of high zinc levels on preterm birth induced by mercury exposure during pregnancy: A birth cohort study in China

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ABSTRACT

Objective: The aims of our study were to determine whether prenatal mercury levels are associated with the risk of preterm birth (PTB) and whether high maternal serum zinc (Zn) levels alleviate any negative effects of maternal mercury (Hg) exposure regarding PTB.

Methods: Serum concentrations of Zn and Hg were measured in 3025 pregnant women from the Ma'anshan Birth Cohort. Before the collection of blood samples, they underwent examinations via the completion of questionnaires. The delivery records of the women were obtained from a series of medical records. We divided the study population into tertiles according to the participants' Hg levels: the low-Hg group (the first tertile, < 0.30 μg/L), the medium-Hg group (the second tertile, 0.30–0.43 μg/L) and the high-Hg group (the third tertile, ≥0.43 μg/L). The associations of Hg exposure with both the risk of PTB and gestational age (weeks) at birth were estimated using a binary logistic regression model and multivariable linear regression analysis, respectively. Afterwards, we conducted a repeated analyses test after the participants were stratified according to their Zn levels, using the 75th percentile division method.

Results: Overall, the medians and the interquartile ranges of Hg and Zn in the second trimester were 0.36 (0.27, 0.48) μg/L and 812.34 (731.26, 896.59) μg/L, respectively. Hg levels were associated with PTB [adjusted odds ratio (OR) and 95% confidence interval (95% CI): 1.91 (1.17, 3.12) for the third tertile vs. the first tertile of the serum Hg levels]. In the stratification analysis of the participants in the low-Zn group, the high-Hg group exhibited a significant odds ratio of PTB [adjusted OR (95% CI): 1.87 (1.08, 3.24)], compared to the low-Hg group. However, in the participants from the high-Zn group, the high-Hg group exhibited a non-significant OR of PTB [adjusted OR (95% CI): 2.32 (0.73, 7.42)]. In the multivariate linear regression analysis, gestational age (weeks) at delivery was significantly and inversely associated with the ln-transformed Hg concentrations [adjusted β (95% CI): −0.16 (−0.26, −0.06)]. Similarly, after the stratification analysis in the high-Zn group, there were no significant associations between PTB and the Hg levels [adjusted β (95% CI): −0.12 (−0.33, 0.09)].

Conclusion: Prenatal Hg exposure adversely affected PTB, and high Zn levels alleviate this effect, which indicates that a more stringent control of Hg and a sufficient intake of Zn are necessary to help birth outcomes.

1. Introduction

Mercury (Hg) exists through various natural and anthropogenic sources in the environment. Human activities contribute to Hg levels in the environment during mining, manufacturing and various other processes [1,2]. Moreover, dental amalgams, which are widely used materials in the field of dentistry around the world, are also major sources of Hg contamination [3]. In some cases, the use of skin creams and traditional herbal medicines may lead to additional Hg exposure [4]. Additional exposures to Hg in humans occur via diet, especially with long-lived fish at the top of the food chain [5]. Previous studies have demonstrated that foetal Hg levels are higher than maternal levels because Hg can easily pass through the placenta [6]. Accumulating evidence has demonstrated a significant correlation between moderate levels of gestational Hg exposure and incidences of neurological diseases [7]. An earlier study has shown that during late pregnancy, high Hg levels in the maternal blood are associated with an increased risk of lower birth weights [8]. One large, community-based study indicated

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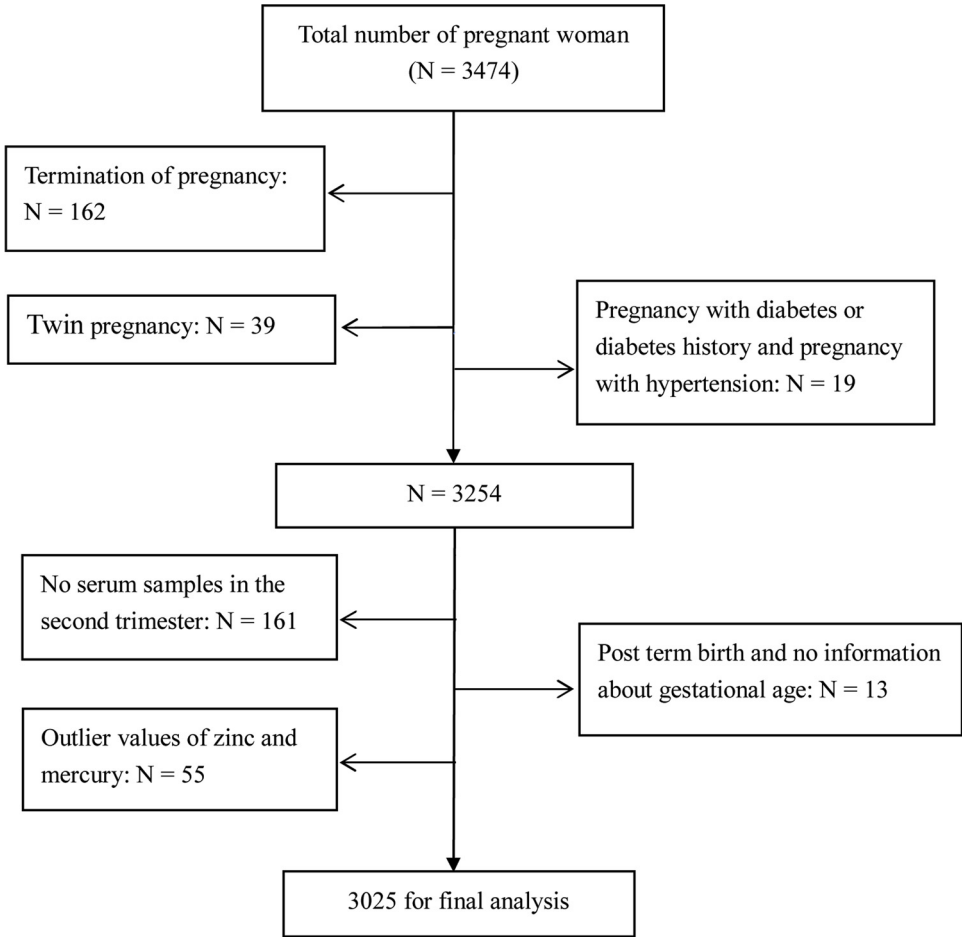

Fig. 1. Flow chart of participant inclusion and exclusion of our study.

that low-to-moderate gestational Hg exposure increased the risk of preterm births (PTBs) [9]. According to a study of Inuit pregnant women, a negative correlation between umbilical cord blood Hg levels and pregnancy duration was demonstrated [10].

Zinc (Zn) is a structural constituent that is essential for cell growth and cell differentiation, and it can be found in various tissues [11]. The homeostasis of Zn is maintained through strictly regulated mechanisms of uptake, storage and secretion, and it is critical for life and embryonic development [12]. Maternal low levels of Zn, which are associated with a variety of adverse pregnancy outcomes, have been examined in previous studies and are associated with such outcomes as stillbirth, abortion, congenital anomalies and foetal neural tube defects [12–15]. Additionally, in several epidemiological studies, the Zn insufficiency in mothers during pregnancy, which is associated with foetal growth restriction, has also been investigated [16,17]. Furthermore, both small and large birth cohort studies have demonstrated that low levels of serum Zn during pregnancy increased the risk of PTBs [18,19]. Recently, a meta-analysis demonstrated that maternal Zn supplementation during pregnancy resulted in a relative 14% reduction in PTBs [20]. However, according to an epidemiological study from Iran, there was no significant association between maternal serum Zn levels and birth outcomes, including weight, height and head circumference [21].

Several experiments on young rats have demonstrated that Hg can be detoxified by Zn treatment [22–27]. Until now, there has been a lack of evidence from birth cohort studies concerning the protective effects of Zn against PTBs that are caused by Hg exposure during pregnancy. At present, the aims of our study are to determine: (a) whether prenatal Hg levels are associated with a risk of PTB, and (b) whether maternal serum Zn levels can alleviate any negative effects of maternal Hg, in regard to PTB.

2. Materials and methods

2.1. Study design

The present study was based on the Ma'anshan Birth Cohort Study (the MABC study), which was conducted to evaluate the impacts of maternal exposure on adverse pregnancy outcomes, including foetal health and the growth of children. The study complied with the principles of the Declaration of Helsinki and the ethics committee Anhui Medical University provided the ethics approval for this study (No: 20160270). All of the participants signed written informed consents in this study. A total of 3474 eligible participants, who were over 18-years-old with gestational ages of less than 14 weeks, participated in our study from May 2013 to September 2014. All of the participants visited the Ma'anshan Maternal and Child Health Care (MCH) Center for their first antenatal care visit, and they were willing to attend specified interviews both during their pregnancies (three times) and after their deliveries. After enrolment, and at each specified interview, the participants were required to complete a structured questionnaire to collect data concerning demographic information, and blood samples were also provided by the patients. Besides, antenatal care and delivery record from a series of medical records were collected from trained investigator, midwife or obstetrician [28]. In this study, among the 3474 participants, 2 cases of ectopic pregnancies, 120 spontaneous abortions, 30 therapeutic abortions, 10 stillbirths, 39 twin pregnancies, 13 pregnancies with diabetes or a history of diabetes, 6 pregnancies with hypertension and 161 pregnancies with no available maternal sera

Table 1
Characteristics of study participants according to gestational age groups.

Characteristics	N	Gestational age		*P*-value
		Premature birth (N = 126, 4.2%)	Full term birth (N = 2899, 95.8%)	
Age (y, means ± SD)	3025	27.40 ± 4.34	26.59 ± 3.57	0.013
≤24 [*n* (%)]	885	26 (2.9)	859 (97.1)	0.084
25–29 [*n* (%)]	1603	73 (4.6)	1530 (95.4)	
≥30 [*n* (%)]	537	27 (5.0)	510 (95.0)	
Pre-pregnancy BMI (kg/m^2, means ± SD)	3025	21.78 ± 3.60	20.81 ± 2.75	< 0.001
< 18.5 [*n* (%)]	570	20 (3.5)	550 (96.5)	< 0.001
18.5–24.9 [*n* (%)]	2094	74 (3.5)	2020 (96.5)	
≥25 [*n* (%)]	361	32 (8.9)	329 (91.1)	
Educational [*n* (%)]				
Middle school or below	601	31 (5.2)	570 (94.8)	0.568
High school	683	25 (3.7)	658 (96.3)	
Junior college	935	38 (4.1)	897 (95.9)	
University or above	806	32 (4.0)	774 (96.0)	
Monthly household income (yuan) [*n* (%)]				
or less	799	40 (5.0)	759 (95.0)	0.218
2500–3999	1296	55 (4.2)	1241 (95.8)	
4000 or more	930	31 (3.3)	899 (96.7)	
Gravidity [*n* (%)]				
1	1676	72 (4.3)	1604 (95.7)	0.693
2	866	32 (3.7)	834 (96.3)	
≥3	483	22 (4.6)	461 (95.4)	
Parity [*n* (%)]				
Primipara	2692	113 (4.2)	2579 (95.8)	0.885
Multipara	333	13 (3.9)	320 (96.1)	
Smoking [*n* (%)]				
Yes	128	10 (7.8)	118 (92.2)	0.065
No	2897	116 (4.0)	2781 (96.0)	
Newborn sex [a] [*n* (%)]				
Boys	1540	75 (4.9)	1465 (95.1)	0.056
Girls	1482	51 (3.4)	1431 (96.6)	

Abbreviations: BMI, body mass index; SD, standard deviation.
[a] Missing data (N = 3).

in the second trimester were excluded. Afterwards, we further excluded participants in cases of post-term birth (n = 11) or a lack of information concerning their gestational ages (n = 2). In addition, according to the method described in previous articles [29,30], box plots were used to identify outlier values. The levels of the outliers were calculated by using a mathematical formula, and they were greater than the 75th percentile of Zn and Hg plus 3× the interquartile range (IQR). Subsequently, we excluded the outlier values from this analysis. These outliers that were included or excluded may have slightly changed the estimates, but they did not affect the significance of the results. To provide valid estimates, we excluded the Zn outlier values (Zn ≥ 1394.691 μg/L, n = 12) and the Hg outlier values (Hg ≥ 1.169 μg/L, n = 43). Eventually, 3025 mother-and-singleton pairs remained for the final analyses (Fig. 1).

2.2. Serum concentrations measurement

Maternal fasting blood samples were collected at approximately 26 weeks in the morning, typically between 7 AM and 10 AM, and were poured into metal-free plain tubes (Anhui Heer Biomediacal Laboratory Science & Technology Co. Ltd, China). After being centrifuged at 3500 rpm for 15 min (low speed centrifuge, KDC-40, Anhui USTC Zonkia Scientific Instruments Co. Ltd, China), the serum samples were stored in metal-free polypropylene (PP) tubes and stored at −80 °C until further analyses. The concentrations of the serum elements were measured by using inductively coupled plasma mass spectrometry (ICP-MS) (Perkin Elmer NexION 350X, Shelton, CT, USA). Briefly, the serum was diluted 1:25 with a diluent containing 1% double-distilled HNO3 (Suzhou, China) and 0.05% Triton®X-100 (Sigma Ultra Grade). We selected Hg^{202} and Zn^{66} as the isotopes of our targeted analytes. The limits of detection (LOD) of the instrument for Hg and Zn were 0.0014 μg/L and 0.0674 μg/L, respectively. No specimens of the Zn levels were below the LOD, and two samples of the Hg levels were below the LOD, which were replaced with LOD/√2. The precisions of the Hg and Zn inter-day and intra-day determinations were 3.89% and 16.72%, and 1.03% and 2.95%, respectively. The recovery rates of Hg and Zn were 88.45% and 97.40%, respectively. The maternal serum levels of selenium (Se^{78}), lead (Pb^{208}), arsenic (As^{75}) and cadmium (Cd^{111}) were also measured as covariates. All of the samples were tested three times, and we used the average of these 3 tests for further analyses. For quality controls, the certified reference materials from Seronorm were used to perform the daily quality controls (Billingstad, Norway, LOT: 1309438). The details of the analytical methods for the serum metals have already been reported in our previous studies [31].

2.3. Outcome variables

We divided the subjects into two groups, according to the gestational ages. PTB was defined as infant birth that occurred before 37 weeks of gestation. Gestation at delivery, from 37 weeks 0 days to 41 weeks 6 days, was defined as full-term birth. Gestational ages and dates of delivery were estimated on the basis of the last set of menstrual period data. In consideration of the reliability of the last recalled menstruation and pregnant woman with irregular menstruation, ultrasound data were instead used if the interval of these two dates was longer than one week [32].

2.4. Confounding factors

Well-trained interviewers collected the information of the participants, including maternal age, weight, height, demographic data, economic status, education, smoking status and alcohol consumption. We calculated pre-pregnancy BMI, which was based on the first available measured maternal height and self-reported prepregnancy weight.

Potential confounding factors that may influence the association between the maternal Hg levels during pregnancy and PTB were listed as follows: maternal age (≤24 years, 25–29 years, ≥30 years), pre-pregnancy BMI (< 18.5 kg/m^2, 18.5–24.9 kg/m^2, ≥25.0 kg/m^2), average monthly income (2499 or less yuan, 2500–3999 yuan, 4000 or more yuan), gravidity (1, 2, ≥3), parity (primipara and multipara), education (middle school or below, high school, junior college, university or above), smoking (yes or no) and new-born sex (boys or girls). Due to the influence of the traditional Chinese culture, few women will drink alcohol during pregnancy; thus, we did not consider alcohol to be a confounding variable in the statistical analysis [33]. In addition, maternal serum selenium, serum lead, serum arsenic and serum cadmium concentrations were also considered to be confounding factors [34].

2.5. Statistical analyses

Serum concentrations of Hg and Zn were expressed as μg/L. Serum Hg and Zn levels in the second trimester were not normally distributed. Thus, the concentrations of serum Hg and Zn were expressed as medians and IQRs. We modelled Hg levels as natural log-transformed (ln-Hg) values, after which the data presented as an approximately normal distribution. In this study, except for maternal age and pre-pregnancy BMI (which were both indicated as continuous variables and categorical variables for analysis), the other variables were considered to be classified variables for the analysis. First, the analyses of the maternal and new-born characteristics, according to the gestational age groups, were performed via t-tests for the normally distributed continuous variables and via Chi-square tests for the classified variables. The comparisons of the maternal serum Hg and Zn concentrations, according to the

Table 2
Maternal serum mercury (Hg) and zinc (Zn) levels according to participants' characteristics.

Characteristics	N (%)	Maternal serum Hg level		Maternal serum Zn level	
		Median (IQR)	*P*-value	Median (IQR)	*P*-value
Age (years)					
≤24	885 (29.3)	0.35 (0.25, 0.47)	0.002	811.48 (732.65, 894.66)	0.826
25–29	1603 (53.0)	0.36 (0.27, 0.48)		812.94 (732.06, 897.11)	
≥30	537 (17.8)	0.37 (0.27, 0.51)		812.34 (730.20, 897.92)	
Pre-pregnancy BMI (kg/m^2)					
< 18.5	570 (18.8)	0.37 (0.27, 0.48)	0.151	804.15 (726.19, 895.10)	0.007
18.5–24.9	2094 (69.2)	0.36 (0.26, 0.48)		810.04 (730.48, 893.20)	
≥25	361 (11.9)	0.37 (0.27, 0.50)		837.83 (744.67, 915.12)	
Educational					
Middle school or below	601 (19.9)	0.36 (0.27, 0.49)	0.003	810.96 (729.08, 899.13)	0.770
High school	683 (22.6)	0.35 (0.25, 0.48)		813.66 (732.66, 900.84)	
Junior college	935 (30.9)	0.35 (0.26, 0.46)		805.27 (733.83, 893.10)	
University or above	806 (26.6)	0.38 (0.27, 0.51)		821.00 (729.08, 895.80)	
Monthly household income (yuan)					
2499 or less	799 (26.4)	0.35 (0.25, 0.46)	0.005	806.16 (730.17, 897.43)	0.615
2500–3999	1296 (42.8)	0.36 (0.27, 0.49)		810.56 (730.79, 898.05)	
4000 or more	930 (30.7)	0.37 (0.27, 0.50)		820.82 (733.03, 895.62)	
Gravidity					
1	1676 (55.4)	0.36 (0.26, 0.48)	0.569	815.91 (735.56, 902.66)	0.043
2	866 (28.6)	0.37 (0.26, 0.49)		805.47 (724.27, 889.35)	
≥3	483 (16.0)	0.36 (0.27, 0.48)		813.36 (731.24, 888.07)	
Parity					
Primipara	2692 (89.0)	0.36 (0.26, 0.48)	0.053	810.56 (730.28, 895.83)	0.068
Multipara	333 (11.0)	0.38 (0.28, 0.50)		833.05 (743.08, 905.21)	
Smoking					
Yes	128 (4.2)	0.38 (0.26, 0.53)	0.451	788.94 (713.21, 892.94)	0.163
No	2897 (95.8)	0.36 (0.26, 0.48)		812.94 (732.59, 897.26)	
Newborn sex [a]					
Boys	1540 (51.0)	0.36 (0.26, 0.48)	0.363	813.41 (731.78, 890.64)	0.675
Girls	1482 (49.0)	0.36 (0.26, 0.49)		810.93 (731.21, 901.25)	

Abbreviations: BMI, body mass index; IQR, interquartile range.
[a] Missing data (N = 3).

Table 3
Distribution of maternal serum Hg and Zn concentrations (μg/L) in the second trimester.

	Percentile					Maximum
	P_5	P_{25}	P_{50}	P_{75}	P_{95}	
Hg	0.16	0.27	0.36	0.48	0.77	1.65
Zn	633.31	731.26	812.34	896.59	1056.04	1391.01

characteristics of the study participants, were performed via non-parametric analyses including Kruskal-Wallis H test and Mann-Whitney U test.

Afterwards, we divided the subjects into three groups, based on the tertiles of the Hg levels: the low-Hg group (the first tertile, < 0.30 μg/L), the medium-Hg group (the second tertile, 0.30–0.43 μg/L) and the high-Hg group (the third tertile, ≥0.43 μg/L). Subsequently, in order to more precisely explore the relationship between Hg concentrations and PTBs, a binary logistic regression model was conducted to estimate the odds ratios (ORs) and the 95% confidence intervals (95% CIs). Furthermore, we also conducted multivariate linear regression models to identify the association between the ln-transformed serum Hg levels in the second trimester and the gestational ages (weeks) at the time of delivery. Model 1 included the following covariates: maternal age, pre-pregnancy BMI, maternal education, monthly household income, gravidity, parity, smoking and new-born sex. Model 2 also included the previously mentioned covariates, as well as serum selenium, lead, arsenic and cadmium concentrations in the second trimester. Afterwards, all of the previous analyses were repeated after Zn was stratified into two groups by using the 75th percentile division method: the low-Zn group (Zn < P_{75}, which indicates Zn < 896.59 μg/L) and the high-Zn group (Zn ≥ P_{75}, which indicates Zn ≥ 896.59 μg/L). We used the

Table 4
ORs (95% CIs) of preterm birth association with concentrations of Hg and Zn in the second trimester.

Concentrations (μg/L)	Preterm birth [a]			
	N (%)	Crude model OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Hg				
Low (N = 1008)	28 (2.8)	1.00	1.00	1.00
Medium (N = 1009)	45 (4.5)	1.63 (1.01, 2.64) *	1.62 (1.00, 2.62)	1.62 (0.99, 2.64)
High (N = 1008)	53 (5.3)	1.94 (1.22, 3.10) *	1.93 (1.21, 3.09) *	1.91 (1.17, 3.12) *
Zn				
< P_{75} (N = 2269)	95 (4.2)	1.00	1.00	1.00
≥ P_{75} (N = 756)	31(4.1)	0.98 (0.65, 1.48)	0.95 (0.63, 1.45)	0.90 (0.57, 1.38)

Abbreviations: low, the first tertile; medium, the second tertile; high, the third tertile; OR, odds ratio; CI, confidence interval.
Model 1: Adjusted for maternal age, pre-pregnancy BMI, maternal education, monthly household income, gravidity, parity, smoking, newborn sex. Model 2: Adjusted for the covariates mentioned above as well as serum selenium, serum lead, serum arsenic and serum cadmium concentrations in the second trimester.
[a] The reference group was full-term birth.
* *P* value < 0.05.

Statistical Package for Social Sciences statistical software (version 10.0, SPSS UK Ltd., Surrey, UK) for all of the statistical analyses. The results were considered statistically significant if two-tailed P values were less than 0.05.

Table 5
ORs (95% CIs) of preterm birth association with the tertiles of Hg after Zn stratificated into two groups in the second trimester.

Hg (μg/L)	Preterm birth [a]			
	N(%)	Crude model OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Zn < P_{75}				
Low	24 (2.9)	1.00	1.00	1.00
Medium	36 (4.7)	1.67 (1.00, 2.83)	1.69 (1.00, 2.87)	1.69 (0.99, 2.88)
High	35 (5.2)	1.90 (1.10, 3.16) *	1.90 (1.11, 3.22) *	1.87 (1.08, 3.24) *
Zn ≥ P_{75}				
Low	4 (2.3)	1.00	1.00	1.00
Medium	9 (3.7)	1.60 (0.49, 5.30)	1.57 (0.47, 5.24)	1.54 (0.46, 5.20)
High	18 (5.3)	2.40 (0.79, 7.16)	2.38 (0.78, 7.29)	2.32 (0.73, 7.42)

Abbreviations: low, the first tertile; medium, the second tertile; high, the third tertile; OR, odds ratio; CI, confidence interval.
Model 1: Adjusted for maternal age, pre-pregnancy BMI, maternal education, monthly household income, gravidity, parity, smoking, newborn sex. Model 2: Adjusted for the covariates mentioned above as well as serum selenium, serum lead, serum arsenic and serum cadmium concentrations in the second trimester.
[a] The reference group was full-term birth.
* P value < 0.05.

Table 6
The associations between gestational age and maternal serum ln-transformed Hg (μg/L) in all participants and participants with different serum Zn levels.

	Gestational ages (weeks)			
	N	Crude model β (95% CI)	Model 1 β (95% CI)	Model 2 β (95% CI)
All				
Continous ln-Hg	3025	−0.16 (−0.25, −0.07) **	−0.14 (−0.24, −0.05) **	−0.16 (−0.26, −0.06) **
Zn < P_{75}				
Continous ln-Hg	2269	−0.18 (−0.29, −0.07) **	−0.17 (−0.28, −0.06) **	−0.17 (−0.28, −0.06) **
Zn ≥ P_{75}				
Continous ln-Hg	756	−0.08 (−0.27, 0.12)	−0.05 (−0.25, 0.14)	−0.12 (−0.33, 0.09)

Model 1: Adjusted for maternal age, pre-pregnancy BMI, maternal education, monthly household income, gravidity, parity, smoking, newborn sex. Model 2: Adjusted for the covariates mentioned above as well as serum selenium, serum lead, serum arsenic and serum cadmium concentrations in the second trimester.
** P value < 0.005.

3. Results

The PTB rate was 4.2% (126/3025 participants) in our study among the 3025 eligible participants. The characteristics of the mother-and-singleton pairs, according to the gestational age groups, are shown in Table 1. The gestational age groups were significantly associated with maternal age (continuous variable) and pre-pregnancy BMI (both P values < 0.05). Table 2 shows that the Hg levels were associated with maternal age, pre-pregnancy BMI, maternal educational status and monthly household income (all P values < 0.05). Zn levels were associated with pre-pregnancy BMI and gravidity (both P values < 0.05). The distributions of maternal serum Hg and Zn concentrations in the second trimester are shown in Table 3. The medians (interquartile ranges) of the Hg and Zn levels in the second trimester were 0.36 (0.27, 0.48) μg/L and 812.34 (731.26, 896.59) μg/L, respectively.

Table 4 shows the crude and adjusted ORs of PTB with maternal serum Hg levels in the second trimester. In the crude model, the risks of PTBs in the medium-Hg group and the high-Hg group were 1.63-fold (95% CI: 1.01, 2.64) and 1.94-fold (95% CI: 1.22, 3.10) higher than the risk in the low-Hg group. After adjusting for all of the confounding factors, the association was still statistically significant [adjusted OR (95% CI): 1.91 (1.17, 3.12) for the high-Hg group vs. the low-Hg group]. Furthermore, as shown in Table 4, after adjustments for all of the potential confounding factors, no significant association was observed between the serum Zn levels and PTB in the multiple logistic regression analyses.

As shown in Table 5, we conducted repeated analyses after the participants were stratified by their Zn levels (Zn < P_{75} and Zn ≥ P_{75}). In the low-Zn group, the high-Hg group exhibited a significant odds ratio of PTB [adjusted OR (95% CI): 1.87 (1.08, 3.24)], compared with the low-Hg group. However, in the high-Zn group, although an increased odds ratio was observed for PTB, the association was not significant.

Table 6 shows the regression β coefficients and 95% CIs for gestational age (weeks) that was associated with ln-Hg in the maternal serum. We observed that gestational age at delivery was significantly and inversely associated with the ln-Hg concentrations [adjusted β: −0.16-weeks (95% CI: −0.26, −0.06)]. After we stratified the participants by their Zn levels, for the participants in the low-Zn group, a similar negative association of PTB with the maternal serum ln-Hg levels was observed [adjusted β (95% CI): −0.17(−0.28, −0.06)]. In the high-Zn group, there were no significant associations between ln-Hg levels and the gestational age.

4. Discussion

The PTB rate was 4.2% in our study, whereas the average global PTB rate is 11.1% [35], which is similar with the rates reported from two other studies in China (3.9% and 3.3%) [36,37] as well as a study in the Netherlands (4.4%) [38]. Several possible reasons for explaining the low preterm birth rate of this study are that: a) most participants in our study were urban residents (61.0%), and they were relatively wealthy; and b) we had excluded 39 cases of twin birth before we performed the analyses. The World Health Organization (WHO) has briefly stated that PTB is an extremely important perinatal health problem around the world and is related to infant mortality and a series of subsequent health problems [39–42]. The findings of our study indicated that gestational Hg exposure is related to PTB and shorter gestational lengths. Furthermore, in this study, we also observed that high Zn levels (in our study, high Zn levels indicated Zn concentrations ≥896.59 μg/L, which are relatively high in the reference Zn range (700–1100 μg/L) for pregnant women [43]), were associated with a significant reduction of the risk of PTB.

The exact mechanism of how gestational Hg exposure affects PTB is not yet clear, but previous studies have shown that Hg exposure can lead to PTB via inflammation and oxidative stress. It has been demonstrated that oxidative stress or an imbalance of oxidative/antioxidant activity in placental tissue may be the key factor for the occurrence of placental-related diseases [44]. In addition, Hg exposure can accelerate the formation of free radicals and can cause lipid peroxidation in the body [45]. Hg-induced oxidative stress may interfere with normal foetal growth in utero via effects on placental function. In a population study, prenatal exposure to high concentrations of methylmercury (MeHg) was demonstrated to be associated with a reduction in foetal fibronectin phosphorylation at the placental side [46]. Several studies have suggested that foetal birth weight loss may be associated with changes in the placenta that are caused by toxic metal exposure and that toxicity of metal may regulate placental function and interfere with the transport of nutrients from the maternal side to the foetus [47]. Previous studies have reported that the decrease in endogenous antioxidant enzyme activities of glutathione reductase and glutathione peroxidase can lead to the further enhancement of oxidation-promoting effects in toxicity that is induced by Hg. Additionally, some studies have shown that methylmercury inhibits glutamic acid transport in human placental tissues [48]. Glutamate, which plays a key role in metabolic pathways, is an amino acid and is an important

nutrient for maintaining foetal intrauterine growth [49].

However, previous studies have provided little evidence of the protective effect of serum Zn levels during pregnancy against the effect of Hg on PTB. These two elements may be mutually antagonistic, in terms of absorption, metabolism and accumulation. According to a previous study, Zn has an antagonistic effect against Hg toxicity because Zn can increase the levels of hepatic metallothionein [50]. Moreover, several experiments on young rats have demonstrated that Hg can be detoxified by Zn [22–27]. Zn, which is a nutrient, may be beneficial for the full development of pregnant women and foetuses, thereby improving observed foetal development [12]. Previous studies have found that the oral administration of Zn could ameliorate the restriction of intrauterine foetal growth that is induced by lipopolysaccharide [51]. Previous animal experiments have demonstrated that Zn supplementation can be used to effectively treat heavy metal-induced oxidative stress. Zn is involved in the synthesis of molecules that are rich in sulfhydryl groups, including metallothionein and reduced glutathione, which have been demonstrated to antagonize the toxicity of Hg [52,53]. Furthermore, earlier animal studies have indicated that Zn supplementation serves an important role in antioxidation and the offsetting of any negative effects of Fas ligands and caspase-3 activation that are induced by TNF-α and cytochrome C. Furthermore, the stimulation of caspase-3, which is involved with apoptosis, was inhibited [54,55]. A previous study has demonstrated that co-administration of zinc sulfate and Hg caused a reduction in Hg accumulation in the placenta. Moreover, zinc sulfate also contributes to better foetal development and reduced S100B expression. S100B expression can block Ca^{2+} apoptosis signalling and is likely to be associated with Zn protection in foetal rats against growth retardation that is induced by maternal Hg exposure [56]. Therefore, further studies need to be performed to elucidate the underlying mechanisms.

This study had several strengths. First, this study was based on a large sample cohort study with extensive reliable data sources that were collected from face-to-face interviews and medical records. Second, as far as we know, this is the first study to explore whether Zn has an antagonistic effect on Hg toxicity in a birth cohort. This study could provide new evidence for further research. Finally, we have controlled various potential confounding variables in our study, including maternal age, pre-pregnancy BMI, maternal education, monthly household income, gravidity, parity, smoking status and new-born sex. We also have controlled for other elements, including serum selenium, lead, arsenic and cadmium levels. However, this study also had several limitations. First, apart from the measurement of the concentrations of different elements, the other studied factors included pregnant woman who self-reported their data, which may lead to an information bias. Furthermore, several residual confounding factors were not taken into account due to related information not being collected, including detailed dietary information, whether patients were taking oral zinc supplements and other information during pregnancy. Second, the traditional multivariable regression method was used to analyse the data and was then adjusted for the possible confounding factors of the mixture components. Consequently, the issue of multicollinearity was ignored. Finally, this study did not explore the antagonistic mechanism of Zn against Hg exposure during pregnancy, which may result in PTB.

5. Conclusion

Our study revealed that Hg exposure increased the risk of PTB during pregnancy, whereas high Zn levels can protect pregnant women against the negative effects that are caused by Hg exposure. The findings of our study are closely related to future public health topics, as they highlight the significance and potential high cost-effectiveness of giving priority to Zn supplementation in pregnant woman who have relatively low Zn baseline statuses. At present, Hg pollution is worsening. Women living in Ma'anshan are exposed to high Hg levels likely through their daily fish-rich diets. Thus, guidelines must be established to improve the health effects of susceptible populations, especially pregnant women with Zn deficiencies.

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